ELECTRON MICROSCOPE STUDY OF EPIDERMOPHYTON FLOCCOSUM*

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Details of bacterial cell structure have been elucidated to some extent through electron microscopy of ultrathin sections. Less has been reported on the application of this relatively new technique to the problem of fungal architecture (1–4) and virtually nothing has been published on thin section studies of dermatophytes, so well recognized clinically as the causative agents in numerous human cutaneous infections. This paper describes exploratory experiments on thin section electronmicroscopy of *Epidermophyton floccosum*.

METHODS

Approximately 1 mm. cubes were cut from the periphery of 14 day colonies of *E. floccosum* grown on solid Sabouraud's medium at room temperature. Some of these were fixed in buffered 1% osmium tetroxide (5) and others in buffered 0.6% potassium permanganate. Still others were fixed in buffered 1% chromate, 1% lead acetate, and 1.5% phosphotungstate. Specimens after fixation for 2–16 hours were dehydrated through the usual alcohol series and embedded in n-butyl methacrylate. Sectioning was performed on a Porter-Blum microtome with glass knives (6). The sections were mounted on carbon films and examined in an RCA EMU microscope fitted with a 25 or 50 μ objective aperture.

OBSERVATIONS AND DISCUSSION

Routine observation soon revealed that osmium tetroxide and potassium permanganate were the only fixatives of those tried that showed any promise at all, and even these appeared to be far from ideal. This was evident from the low percentage of well fixed intact cells found and we resorted to cell selection in obtaining electronmicrographs. Although there may be some justification in this procedure (6) it should be emphasized that the findings are preliminary in nature and must necessarily be confirmed by further study with ever improving technics.

Fig. 1 is a longitudinal section through a hypha of *E. floccosum*, fixed in osmium tetroxide. Although the cytoplasm in this specimen appears to offer little information on structural details, the cell walls are interesting. As prepared in this study the hyphae have a wall thickness of up to 0.5 μ with a cell diameter of 4–6 μ. It is apparent that the cell wall is covered both inside and out, by a thin osmiophilic layer that has been pulled away from the wall in many areas. Whether this layer represents a discrete membrane or an integral part of the wall structure forcibly removed during the preparative procedures remains to be determined. While it contains the same osmiophilic outer coatings, the cross wall also exhibits an additional layer of material, appearing as a broad line, running throughout the center of the cross wall. Although a faint, broken thin line can also be seen in parts of the center of the outer wall, it is always much more pronounced in the cross wall. The difference observed is even more apparent in Fig. 2, where the specimen was fixed in permanganate. Under these conditions the outer wall appears to be quite homogeneous while the septal, or cross wall, consists of numerous membranous or fibrous structures in addition to the well-defined central zone.

Potassium permanganate turned out to be a very interesting cytoplasmic fixative as well. The cytoplasm of *E. floccosum* in permanganate fixation consists of a lightly staining granular area containing what appear to be double membraned vacuoles. These stained uniformly inside. In addition to this lightly staining area there is a darker area, which appears to be concentrated in the periphery of the cytoplasm just inside the cell walls. Its texture is only slightly less granular. The significance, if any, of these granules must await studies with other fixatives, since the tendency of permanganate to impart a granular texture to mammalian tissues has been noted (8). In Fig. 2 the arrows point to what are apparently mitochondria, although details are not too evident in this particular section. They appear to be distributed in both the dark and light areas.

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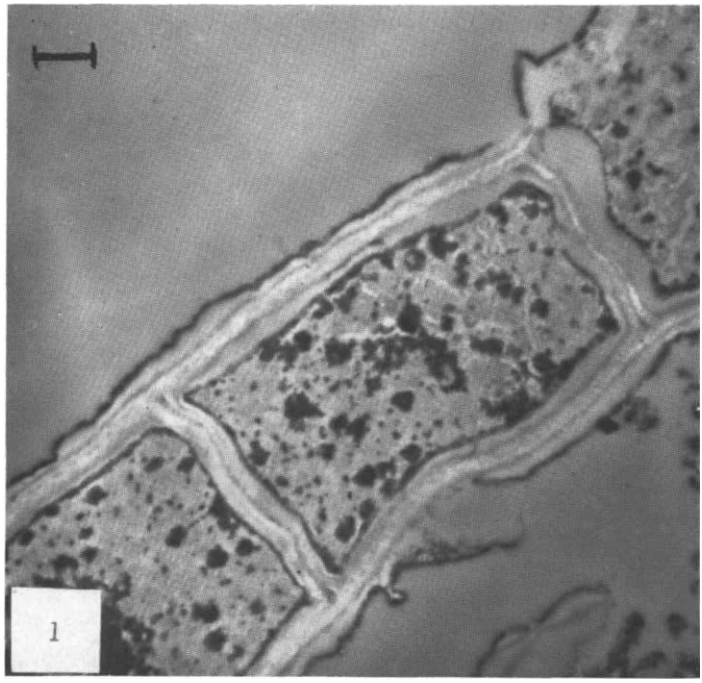

FIG. 1. A longitudinal section through a hypha of *E. floccosum*. Fixed in osmium tetroxide. × 7,940

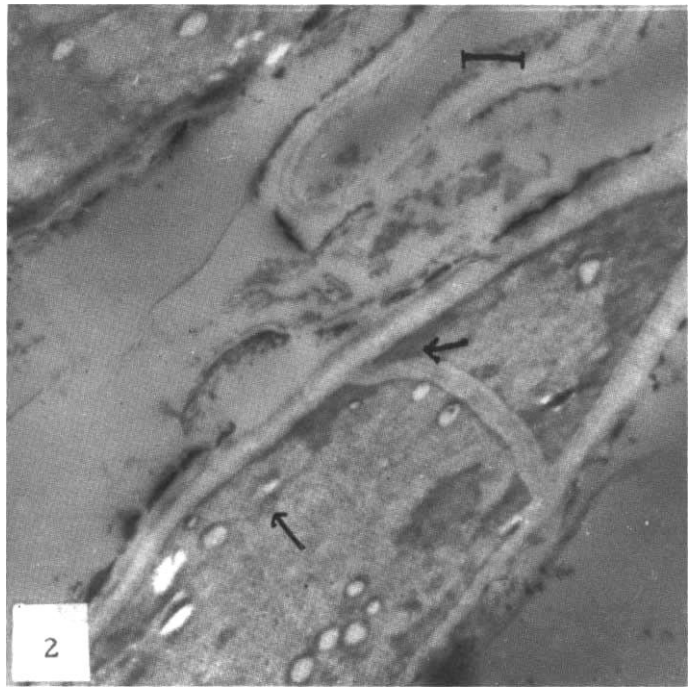

FIG. 2. A diagonal section through a hypha of *E. floccosum*. Fixed in potassium permanganate. × 7,940

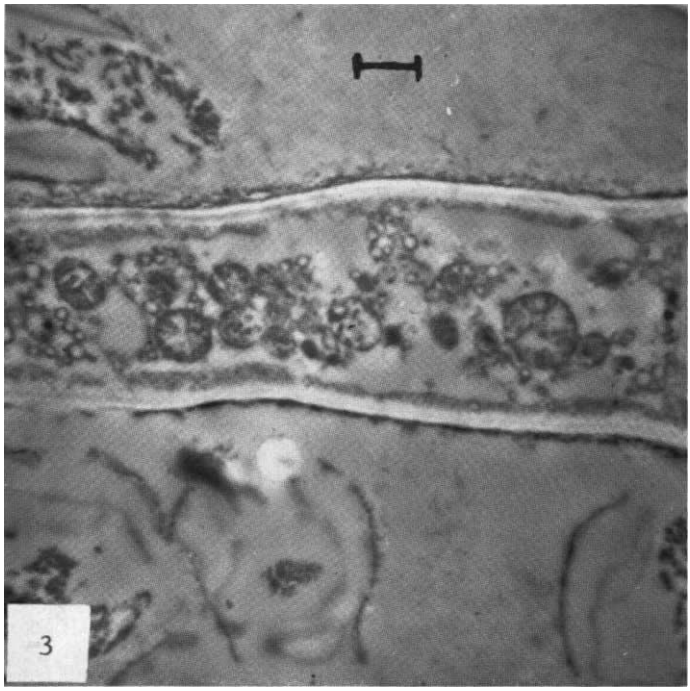

FIG. 3. A longitudinal section through a hypha of *E. floccosum*. Fixed in osmium tetroxide. × 7,940

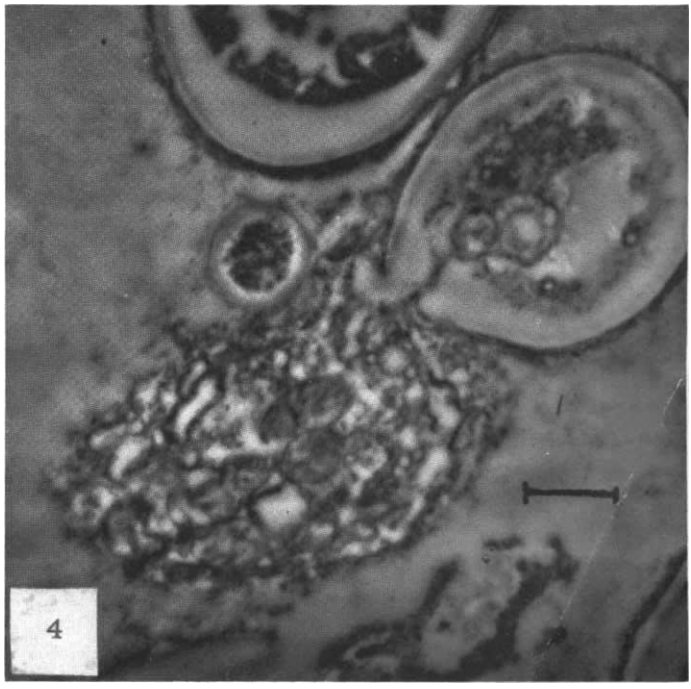

FIG. 4. A cross section through a hypha of *E. floccosum*. Fixed in osmium tetroxide. × 12,700

Figs. 3 and 4 show more clearly the general appearance of these bodies under osmium tetroxide fixation. There is good evidence of a limiting membrane and some internal protrusions not unlike the familiar cristae seen in mitochondria from many sources. In general they are more spherical than rod-shaped. In Fig. 4 the contents of a hypha have found their way out into the surrounding medium either prior to fixation or as a result of fixing and embedding. Nevertheless, the mitochondria are similar to those within the intact cells.

There was no evidence of anything resembling a discrete nucleus or even anything that might be called a nuclear apparatus. Discrete nuclei have been found in other fungi imperfecti, namely *C. immitis* (1, 9). There is a paucity of information on the subject of nuclear bodies in the dermatophytes but the consensus seems to be that nuclear stains used in light microscopy are spread throughout the organism with no clear zones of concentration, unless it is in submicroscopic particles. This preliminary study throws no light on this concept of the nuclear material. The numerous small particles seen in Fig. 3 could conceivably represent such particles. However, we attach no significance to them at this time for two reasons: 1. the general washed-out appearance of the cytoplasmic background in this particular section indicates that they could be artifacts and 2. the complete absence of such particles in the permanganate fixed sections. This fundamental question could perhaps be answered by a combination of improved methods in electron microscopy together with the resourceful application of the methods of modern biochemistry.

SUMMARY

Some morphological features of *E. floccosum* as seen in osmium tetroxide and permanganate fixed thin sections are described. The findings include: 1. lack of a discrete nucleus, 2. more complex structure of the septal wall as compared to the outer wall, and 3. the presence in the cytoplasm of bodies similar to mitochondria.

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